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Distribution of genotypes and antibiotic resistance genes among invasive *Streptococcus agalactiae* (group B streptococcus) isolates from Australasian patients belonging to different age groups

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ABSTRACT

Serotype distribution and antibiotic resistance (AR) among group B streptococci (GBS) affect GBS disease prevention strategies, but vary among patient groups. A multiplex PCR-based reverse line blot (mPCR/RLB) hybridisation assay was used to compare the distributions of GBS serotypes, serotype III subtypes and AR-associated genes among 666 invasive isolates from 663 patients, divided into five age groups: infants, early-onset (EO; 0–6 days) and late-onset (LO; 7–90 days); children (aged 3 months to 14 years); women of childbearing age (WCBA; aged 15–45 years); and other adults (males aged >15 years; females aged >45 years). Serotypes Ia and V and serosubtype III-1 accounted for 60% of infections. Serosubtype III-2, which corresponds to a virulent clone belonging to sequence type (ST)17, was relatively uncommon overall (7%), but was associated strongly with LO infant infections, in which it was significantly more common than in adult infections (25/104 (24%) vs. 9/392 (2%), $p < 0.0001$) or in EO infections (25/104 (24%) vs. 14/155 (9%), $p < 0.005$). Erythromycin resistance genes were found in 8% of all isolates (*ermB* 3%, *ermA* 2.5% and *mefA/E* 2%), in 11–15% of isolates of serotypes II and V and subtype III-1, but in none of the isolates of serosubtype III-2 (III-2, 0/49 vs. all others, 54/618 (9%), $p < 0.04$). In summary, the virulent serosubtype III-2 was associated strongly with LO infant GBS infection, but was less likely than other serotypes or serosubtype III-1 to carry AR genes.

Keywords Antibiotic resistance, distribution, group B streptococci, mPCR/RLB, serotypes, *Streptococcus agalactiae*

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INTRODUCTION

Group B streptococci (GBS; *Streptococcus agalactiae*) are a common cause of neonatal and obstetrical sepsis, and are an increasingly important cause of infection in the elderly and in patients with chronic underlying medical conditions [1,2]. Infections in the elderly vary in severity from mild urinary tract infections to life-threatening sepsis and endocarditis [2]. The incidence of neonatal sepsis has fallen significantly over the past decade, partly because of widespread use of intra-partum antibiotic prophylaxis for GBS carriers. Penicillin is the drug of choice for prophylaxis and treatment of GBS disease, with erythromycin and clindamycin recommended for patients with a β-lactam allergy. However, widespread intra-partum antibiotic prophylaxis is likely to contribute to the emergence of antibiotic-resistant GBS [3]. There has been considerable progress in the development of conjugate polysaccharide GBS vaccines [4], the formulation of which depends on the distribution of GBS serotypes. This varies among different patient

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groups, among geographical areas and over time. Surveillance is an important adjunct to vaccine development [5].

Previous reports have described the development and evaluation of multiplex PCR-based reverse line blot (mPCR/RLB) assays for the identification of GBS molecular serotypes, subtypes of molecular serotype (MS) III, and nine antibiotic resistance (AR)-related markers [6,7]. In the present study, these assays were modified and combined into a single mPCR/RLB assay and were used to test a large collection of invasive GBS isolates from Australia and New Zealand. MS III subtypes were also identified on the basis of sequence variation in the *cps* gene cluster [8]. The distribution of molecular serotypes, MS III subtypes and AR markers was compared among five patient groups over a 12-year period to provide important baseline data before the introduction of a GBS vaccine and to inform the clinical management of GBS infections.

MATERIALS AND METHODS

Invasive GBS isolates

Invasive GBS isolates were obtained from 663 patients (605 isolates from blood, 35 from cerebrospinal fluid (CSF), and 23 from joint fluid or other normally sterile sites) between 1994 and 2005. All were serotyped at the Streptococcal Reference Laboratory, Institute of Environmental Science & Research Ltd (ESR), Kenepuru Science Centre, Porirua, New Zealand. Of the 663 isolates, 446 were from New Zealand; these comprised all invasive isolates (excluding 15 that were non-viable) referred to ESR for serotyping between 1994 and 2004 from laboratories throughout New Zealand (population *c.* 4 million). The remaining 217 isolates were from Australia; these comprised all isolates cultured from normally sterile sites (excluding 11 that were non-viable) between 1996 and 2005 at the Centre for Infectious Diseases and Microbiology (CIDM), Westmead, NSW, Australia. The CIDM is a large diagnostic laboratory providing services to a tertiary referral centre and several district hospitals; these provide basic and specialised obstetrical and neonatal services, as well as basic (but not specialist) paediatric services, for a population of *c.* 900 000 in western Sydney.

GBS cases were classified into one of five groups (Table 1). Infants with early-onset (EO) and late-onset (LO) disease were those from whom GBS were isolated in the first 6 days and between 7 and 90 days following birth, respectively [9]. GBS infections in children aged 3 months to 14 years were uncommon and were grouped together. Women of childbearing age (WCBA; aged 15–45 years) were considered separately; 27 women in this group were known to be pregnant or postpartum, and it was assumed that this would also apply to a significant proportion of the remaining 66 women, for whom this information was not available. Other adults included males aged ≥15 years and females aged >45 years. The distribution of isolates among patient groups in each country is shown in Table 1.

Table 1. Distribution into categories of 663 patients with invasive disease caused by group B streptococci (GBS)[a]

Patient category (age)	Sydney (CIDM)[b], *n* (%)	New Zealand (ESR)[c], *n* (%)
Infant: early onset, neonatal (aged <7 days)	44 (20)	109 (25)
Infant: late onset (aged 7–90 days)[d]	13 (6)	91 (20)
Children (aged 3 months to 14 years)	5 (2)	11 (2)
Women of childbearing age (aged 15–45 years)	23 (11)	70 (16)
Other adults (males aged >15 years; females aged >45 years)	133 (61)	164 (37)
Total	218 (100)	445 (100)

[a]Defined as the isolation of GBS from a normally sterile site.
[b]The CIDM (Centre for Infectious Diseases and Microbiology, Westmead, NSW, Australia) provides diagnostic services to a tertiary referral hospital (with specialist obstetrics and neonatal, but no paediatric, services) and several district hospitals in western Sydney.
[c]ESR (Institute of Environmental Science & Research, Pty Ltd, Porirua, NZ) is the main public health reference laboratory in New Zealand.
[d]The proportion of isolates from late-onset cases in Sydney was significantly lower than in New Zealand, because the CIDM does not provide microbiological services to a specialist paediatric facility.

mPCR/RLB

This study combined two mPCR/RLB assays described previously [6,7]. The final assay incorporated one primer pair and two species-specific probes for GBS, for each of its nine serotypes, and for eight AR markers (18 primer pairs and 36 probes). Amplification and hybridisation conditions were as described previously [6,7]. MS III isolates were subtyped on the basis of *cps* sequence variation and the presence of one or more mobile genetic elements [8]. When discrepancies were identified between conventional serotyping and molecular serotyping (as determined by mPCR/RLB), several colonies from each isolate were tested separately by individual serotype-specific PCRs [6,10] and the conventional serotyping tests were repeated, if necessary.

Statistical analysis

SPSS for Windows v.13.0 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Chi-square and Fisher's exact tests were used, as appropriate, to compare differences in the distribution of genetic markers among groups, with $p < 0.05$ considered to be statistically significant.

RESULTS

Comparison of conventional serotyping with molecular serotyping by mPCR/RLB

Seventy (11%) isolates were non-typeable by conventional serotyping; these were divided among the five patient groups as follows: EO, 17; LO, five; children, two; WCBA, eight; and other adults, 37. These initially non-typeable isolates were subsequently assigned by molecular

serotyping to the following types: MS Ia, six (i.e., 4% of the total number of 163 MS Ia isolates identified by conventional serotyping and molecular serotyping); Ib, 17 (20% of 87); II, five (9% of 54); III, 12 (6% of 192); IV, five (25% of 20); V, 18 (14% of 131); VI, five (38% of 13); VII, one (33% of three); and VIII, two (67% of three). The molecular serotypes identified by mPCR/RLB were the same as those assigned previously by conventional serotyping for 590 (99%) of 596 isolates for which both results were available. Six (0.9%) isolates gave discordant results as follows: conventional serotype (CS) Ia/MS V; CS Ib/MS II; CS V/MS III (two isolates); CS II/MS indeterminate; and CS Ib/MS indeterminate.

All 76 isolates with discordant or missing conventional serotyping results were retested using individual serotype-specific PCR and conventional serotyping, which confirmed the mPCR/RLB results for three isolates with previously discordant results; 70 remained non-typeable by conventional serotyping and the mPCR/RLB results were confirmed by serotype-specific PCR. Initial conventional serotyping results were confirmed for the three remaining isolates with discordant results, but serotype-specific PCRs were positive in each case for two serotypes. These isolates were subcultured and several single colonies of each were tested separately using serotype-specific PCRs. Each of these single colony subcultures gave a positive result in only one serotype-specific PCR, and these results were confirmed, using one representative of each serotype, by conventional serotyping. These results indicate that the original isolates were mixed cultures. The mixed cultures were from patients in different age groups and involved various serotypes as follows: WCBA, serotypes Ia and V; EO, serotypes II and III; and other adults, serotypes Ib and V.

Table 2. Distribution of molecular serotypes of group B streptococci according to patient category

MS/msst	Paediatric groups[a], *n* (%)			Adult groups[b], *n* (%)		Total, *n* (%)[c]
	EO	LO	Children	WCBA	Other	
Ia	46 (30)	25 (24)	5 (31)	29 (31)	58 (19)	163 (24)
Ib	19 (12)	14 (13)	1 (6)	15 (16)	38 (13)	87 (13)
II	16 (10)	1 (1)[d]	0	7 (7)	30 (10)	54 (8)
III-1	30 (19)	20 (19)	4 (25)	12 (13)	44 (15)	110 (17)
III-2	14 (9)	25 (24)[e]	1 (6)	5 (5)	4 (1)	49 (7)
III-3	3 (2)	2 (2)	2 (13)	2 (2)	6 (2)	15 (2)
III-4	7 (5)	3 (3)	0	1 (1)	7 (2)	18 (3)
III total[f]	54 (35)	50 (48)	7 (44)	20 (21)	61 (20)	192 (29)
IV	3 (2)	1 (1)	0	2 (2)	14 (5)	20 (3)
V[g]	13 (8)	13 (13)	3 (19)	18 (19)	84 (28)	131 (20)
VI	2 (1)	0	0	2 (2)	9 (3)	13 (2)
VII	0	0	0	1 (1)	2 (1)	3 (0.5)
VIII	1 (1)	0	0	0	2 (1)	3 (0.5)
Total	154 (100)	104 (100)	16 (100)	94 (100)	298 (100)	666 (100)

MS, molecular serotype; msst, molecular serosubtype (MS III only); see text for definition of serosubtype; EO, early onset; LO, late onset; WCBA, women of childbearing age.
[a]Paediatric groups: EO (neonates), aged <7 days; LO (infants), aged 7–90 days; children, aged 90 days to 14 years.
[b]Adult groups: WCBA, aged 15–45 years; other adults, males aged >14 years; females, aged >45 years.
[c]Isolates from three of 663 patients were mixtures of two serotypes; each was treated as a separate isolate.
[d]MS II: LO (1/104; 1%) vs. EO (16/154; 10%), p 0.005; LO (1/104; 1%) vs. all other groups (53/562; 9%), p 0.006.
[e]msst III-2: LO (25/104; 24%) vs. EO (14/154; 9%), p 0.005; LO (25/104; 24%) vs. all other groups (24/562; 4%), p <0.001.
[f]MS III: infants and children (111/274; 41%) vs. adults (81/392; 21%), p <0.001.
[g]MS V: infants and children (29/275; 11%) vs. adults (102/392; 26%), p <0.001; EO (13/155; 8%) vs. WCBA (18/94; 19%), p 0.03.

Distribution of molecular serotypes among patient groups

MS Ia and III were the most commonly identified serotypes among isolates from infants and children (Table 2). MS V was significantly more common (102/392, 26% vs. 29/274, 11%; p <0.001) and MS III was significantly less common (81/392, 21% vs. 111/274, 41%; p <0.001) in adults than in infants and children. For MS III, this difference was attributable mainly to a significantly higher proportion of serosubtype III-2 isolates in the LO group as compared with all other groups (25/104, 24% vs. 24/562, 4%; p <0.001). Serosubtype III-2 was also significantly more common in the LO than in the EO group (25/104, 24% vs. 14/154, 9%; p 0.005). MS II was significantly less common in the LO group than in all other groups combined (1/104, 1% vs. 53/562, 9%; p 0.006) or the EO group (1/104, 1% vs. 16/154, 10%; p 0.005).

Differences between isolates in the EO and WCBA groups were similar to those between the combined paediatric and adult groups; i.e., there were significantly more MS V isolates (13/154, 8% vs. 18/94, 19%; p 0.031) and somewhat, but not significantly, fewer MS III isolates (54/154, 35% vs. 20/94, 21%; p 0.09) in the latter group. There was no significant difference in the distribution of serotypes between WCBA and other adults.

AR markers

The most frequently identified AR marker in all groups was *tetM*, which was present in 560 (84%) isolates (Table 3), most of which (360/560, 64%)

Table 3. Distribution of antibiotic resistance (AR) markers among invasive isolates of group B streptococci according to patient category

AR marker[a,b]	Paediatric groups, *n* (%)			Adult groups, *n* (%)		Total *n* (%)
	EO	LO	Children	WCBA	Other	
tetM	132 (86)	87 (84)	14 (88)	81 (86)	246 (83)	560 (84)
int-Tn	85 (55)	60 (58)	11 (69)	47 (50)	173 (58)	376 (56)
tetO	5 (3)	0	1 (6)	1 (1)	6 (2)	13 (1.9)
ermB	5 (3)	3 (3)	0	2 (2)	12 (4)	22 (3.3)[c]
ermA	5 (3)	0	1 (6)	1 (1)	10 (3)	17 (2.5)[c]
mefA/E	2 (1)	1 (1)	0	1 (1)	11 (4)	15 (2.2)[c,d]
aphA-3	1 (1)	1 (1)	0	1 (1)	5 (2)	8 (1.2)
aad-6	1 (1)	1 (1)	0	2 (2)	7 (2)	11 (1.6)
Total	154 (100)	104 (100)	16 (100)	94 (100)	298 (100)	666 (100)

EO, early onset; LO, late onset; WCBA, women of childbearing age (for definitions, see main text).
[a]AR markers encode resistance to the following antibiotics [7]: *tetM* and *tetO*, tetracycline; *ermA* and *ermB*, macrolides, lincosamine and streptogramin B; *mefA/E*, 14–15-member macrolide antibiotics, including erythromycin; *aphA-3* and *aad-6*, aminoglycosides. *int*-Tn is an integrase gene carried on transposons belonging to the Tn*916* group, which usually carry *tetM*.
[b]None of the differences in frequencies of AR-related genes among patient groups was statistically significant.
[c]Two isolates contained two macrolide resistance genes: one with *ermA* and *ermB*; one with *ermA* and *mefA/E*.
[d]Based on type-specific PCR, eight of 15 *mef*-positive isolates had *mefE* (personal unpublished results).

also contained *int*-Tn. Macrolide resistance genes were found in a small proportion of isolates (52/666; 8%) and each of the three relevant genes was represented: *ermB*, 3.3%, *ermA*, 2.5% and *mefA/E*, 2.3%. One isolate contained both *ermA* and *ermB*, and one contained both *ermA* and *mefA/E*. Based on previous results [7], there is excellent correlation between the presence of any of these genes and phenotypic resistance to erythromycin, and the presence of *ermA* and/or *ermB* and constitutive or inducible resistance to clindamycin. There were no significant differences in the distribution of resistance genes among patient groups (Table 3).

Distribution of AR markers among molecular serotypes

The proportions of isolates with AR markers varied significantly according to MS for all the markers examined, with the exception of *mefA/E* (Table 4). The greatest variation was in the prevalence of *tetM* and *int*-Tn. The majority (214/223; 96%) of *tetM*-positive MS V and serosubtype III-1 isolates also contained *int*-Tn, but this was true of only a small proportion (30/152; 20%) of *tetM*-positive MS Ia isolates ($p < 0.001$). *int*-Tn was found in the absence of *tetM* in only 16 (4%) of 376 isolates, of which a disproportionate number belonged to serosubtype III-2 (ten of 49; 20%). No AR markers were found in 73 (11%) of 666 isolates overall, but this proportion varied from 3% (4/131) in MS V to 69% (9/13) in MS VI (Table 4). Serosubtype III-2 was the only MS/serosubtype in which none of the isolates tested contained any of the five macrolide or aminoglycoside resistance genes, whereas 52 (8%) of 617 isolates belonging to other MS/serosubtypes contained at least one of these genes.

Table 4. Distribution of antibiotic resistance (AR) markers among invasive isolates of group B streptococci according to molecular serotype (MS)

MS	Antibiotic resistance markers, *n* (%)									
	No AR markers	*tetM*	*int*-Tn	*tetO*	*ermB*	*ermA*	*mefA/E*	*aphA-3*	*aad-6*	Total
Ia	7 (4)[a]	152 (93)	32 (20)[b]	1 (1)	1 (1)	0	7 (4)	0	0	163
Ib	19 (22)	68 (78)	57 (66)	0	0	1 (1)	1 (1)	0	1 (1)	87
II	8 (15)	40 (74)	35 (65)	4 (7)	3 (6)	2 (4)	1 (2)	0	0	54
III-1	4 (4)[c]	100 (91)	96 (87)	6 (5)	6 (5)[d]	3 (3)[d]	2 (2)[d]	4 (4)	5 (5)	110
III-2	6 (12)	33 (67)	24 (49)	0	0 (0)[e,f]	0 (0)[e,f]	0 (0)[e,f]	0[f]	0[f]	49
III-3	4 (27)	9 (60)	4 (27)[c]	0	1 (7)	0	2 (13)	0	0	15
III-4	7 (39)	10 (56)	5 (28)	0	1 (6)	0	0	0	0	18
IV	2 (10)	17 (85)	3 (15)	1 (5)	0	2 (10)	0	0	1 (5)	20
V	5 (4)[g]	123 (94)	118 (90)	1 (1)	10 (8)[h]	9 (7)[e]	1 (1)[h]	4 (3)	4 (3)	131
VI	9 (69)	4 (31)	1 (8)	0	0	0	1 (8)	0	0	13
VII	0	3	1	0	0	0	0	0	0	3
VIII	2 (67)	1	0	0	0	0	0	0	0	3
Total	73 (11)	560 (84)	376 (56)	13 (2)	22 (3)	17 (3)	15 (2)	8 (1)	11 (2)	666

[a]No AR markers in Ia (7/163; 4%) vs. all other isolates (66/503; 13%); p 0.004.
[b]*int*-Tn in MS Ia (32/163; 20%) vs. all other isolates (344/503; 68%); p <0.001.
[c]No AR markers in serosubtype III-1 (4/110; 4%) vs. all other isolates (69/556; 12%); p 0.013.
[d]Any macrolide/lincosamine resistance in MS V (28/131, 21%) vs. all other isolates (45/535; 8%); p <0.001.
[e]Any macrolide/lincosamine resistance in serosubtype III-2 (0/49) vs. all other common MS Ia, Ib, II and V and serosubtype III-1 (47/545; 9%); p 0.04.
[f]Apart from serosubtype III-2, all other groups contained some isolates with at least one macrolide or aminoglycoside resistance gene (0/49 vs. 52/617; p 0.04).
[g]No AR markers in MS V (5/131; 4%) vs. all other isolates (68/535; 13%); p 0.007.
[h]Any macrolide/lincosamine resistance in serosubtype III-1 (20/110; 18%) vs. all other isolates (53/556; 10%); p 0.02.

Comparisons between Sydney, Australia and New Zealand

There was a significantly smaller proportion of LO isolates among the isolates from Sydney as compared with New Zealand (Table 1), which reflects the fact that the CIDM does not provide microbiology services to a specialist paediatric centre. Because of this, comparisons between geographical areas were made only within patient groups. There were no significant differences in the distribution of molecular serotypes or AR markers between the two countries (data not shown), except in the EO disease group, for which there was a significantly higher proportion of MS Ib isolates among isolates from New Zealand as compared with those from Sydney (18/110; 16% vs. 1/44; 2%, p 0.03).

Comparison of isolates over time according to country and patient group

Among isolates from Sydney, there were no significant differences between isolates collected in 1996–2000 and those collected in 2001–2005 (data not shown). In New Zealand, there was a significant increase between 1994–2000 and 2001–2005 in the proportion of serosubtype III-1 isolates in the EO, LO and WCBA groups combined (5/69, 7% vs. 42/202, 21%; p 0.03), which was associated with increases in some AR markers. In 1994–2000, only one (1%) of 108 isolates was erythromycin-resistant (*ermA*) as compared with 27/338 (8%) in 2001–2005 (p 0.02); of the latter, 14 (4%) had *ermB*, eight (2%) had *ermA* and five (1%) had *mefA/E*. Aminoglycoside resistance markers were not identified in 1994–2000, but were found in 1% of isolates in 2001–2005 (0/108, vs. 3/338; p 0.33; NS).

Comparison between blood and CSF isolates

Comparison of the distribution of MS/serosubtypes between isolates from blood (n = 608) and CSF (n = 35), with 23 isolates from other sterile sites excluded, revealed that MS III overall, and serosubtypes III-2 and III-3 individually, were significantly more common among CSF isolates (Table 5). A disproportionate number of CSF isolates (20/35; 57%), as compared with blood isolates (83/608; 14%), were from the LO group, which is potentially a confounding factor in the association between serosubtype III-2 and LO disease described above. However, comparison of MS distribution according to patient category for blood isolates showed that only the association between serosubtype III-2 and the LO group remained (serosubtype III-2 in LO, 18/83, 22% vs. serosubtype III-2 in all other groups, 24/526, 5%; p <0.001).

Table 5. Distribution of molecular serotypes/subtypes (MS/serosubtype) of group B streptococci according to site of isolation (blood or cerebrospinal fluid (CSF))

MS or serosubtype	CSF isolates all patients, *n* (%)/LO, *n* (%)[a]	Blood isolates all patients, *n* (%)/LO, *n* (%)[a]
Ia	4 (11)/2 (10)	155 (25)/23 (28)
Ib	7 (20)/3 (15)	76 (13)/11 (13)
II	1 (3)/0	50 (8)/1 (1)
III-1	9 (26)/5 (25)	96 (16)/14 (17)
III-2[b]	7 (20)/7 (35)	42 (7)/18 (22)[c]
III-3[d]	3 (9)/1 (5)	11 (2)/1 (1)
III-4	1 (3)/1 (5)	17 (3)/2 (2)
III total[e]	20 (57)/14 (70)	166 (27)/35 (42)
IV	0/0	19 (3)/1 (1)
V[f]	2 (6)/1 (5)	125 (21)/2 (2)
VI	1 (3)/0	12 (2)/0
VII	0/0	2 (0.3)/0
VIII	0/0	3 (0.5)/0
Total	35 (100)/20 (100)	608 (100)/83 (100)

LO, late onset (see text).
[a]None of the differences between CSF and blood isolates in the LO group was statistically significant.
[b]MS III-2 all patients, CSF 7/35, 20% vs. blood 42/608, 7%; p 0.012.
[c]Blood isolates only, serosubtype III-2 in LO, 18/83, 22% vs. all other groups 24/525, 5%; p <0.001.
[d]MS III-3 all patients, CSF 3/35, 9% vs. blood 11/608, 2%; p 0.011.
[e]MS III (overall) all patients, CSF 20/35, 57% vs. blood 166/608, 27%; p 0.01.
[f]MS V all patients, CSF 2/35, 6% vs. blood 125/608, 21%; p 0.06 (not significant).

DISCUSSION

This is the first comprehensive study in Australasia, and one of the largest overall, of serotype and AR distribution among invasive GBS isolates from all age groups. A convenient mPCR/RLB method was used to characterise the isolates, combining separate assays for identification of molecular serotypes [6] and detection of AR-related genes [7] in GBS. Three (0.5%) of 605 blood cultures were shown to contain two serotypes, as shown by indeterminate mPCR/RLB results, which were resolved by performing serotype-specific PCR and conventional serotyping on subcultures of several individual colonies.

The present study demonstrated or confirmed the following significant findings: (i) a virulent GBS clone, represented by a subtype of serotype III (serosubtype III-2), was associated strongly with LO, but not EO, GBS disease in

infants; (ii) the predominant GBS serotypes (Ia, III, V) causing invasive infection in Australasia were similar to those found in North America and Europe; (iii) there were significant differences in the distribution and incidence of AR between the two major subtypes of MS III, with serosubtype III-1 being significantly more common in all groups except LO infants, and significantly more likely to contain AR genes than was serosubtype III-2; (iv) there were significant differences in the distribution of AR markers among different MS/serosubtypes, with serosubtype III-2 isolates being significantly less likely to contain erythromycin or aminoglycoside resistance genes than all other MS/serosubtypes combined; (v) the low incidence of *int*-Tn in *tetM*-positive MS Ia isolates, as compared with other common MS/serosubtypes, suggested that transmission of *tetM* in MS Ia commonly occurs by a mechanism not involving a transposon of the Tn*916* group; (vi) erythromycin resistance was uncommon among invasive isolates in the region, but has increased in New Zealand over the past 5 years in association with a relative increase in MS V and serosubtype III-1; and (vii) GBS meningitis was more common among the LO infant group than among the other patient groups, with all major MS/serosubtypes being represented among recognised cases of meningitis, but MS III (and specifically serosubtypes III-2 and III-3) being disproportionately represented among CSF isolates as compared with other MS/serosubtypes. However, this does not wholly explain the strong association of serosubtype III-2 with LO disease in infants.

If the assumption that pregnancy is the most common risk-factor for invasive GBS disease in WCBA is correct, the differences in the distribution of serotypes between the EO infant and WCBA groups suggests that the pathogenesis of invasive disease is different in mothers and infants in the first week of life. While this is plausible, and appears to be the case for infants with LO infection, it could also indicate that the WCBA group included a significant proportion of women with non-pregnancy-related risk-factors similar to those in other adults.

Previous studies have reported an association between neonatal sepsis and a virulent GBS clone belonging to multilocus sequence type (ST)17 [11–14] and serotype III. Typically, this virulent strain carries genetic markers, including the group II intron, GBSi1, typical of a pathogenicity island-like region that shares homology with *Streptococcus pyogenes* [14–17]. In a recent study from Oxford, UK [18], ST17 was the only ST identified significantly more frequently among invasive isolates from neonates than among vaginal isolates from pregnant woman. The difference was considerably more marked for LO infants (18/45, 40% vs. 19/190, 10%; OR 6.0; 95% CI 2.6–13.8; p <0.00001) than for EO infants (15/64, 23% vs. 19/190, 10%; OR 2.8; 95% CI 1.2–6.2; p 0.004) isolates. The fact that all ST17 isolates belonged to serotype III, but that many serotype III isolates belonged to other STs (mainly ST19), indicated that the invasiveness of ST17 is not caused solely by the serotype III capsule [18]. Although the present study did not compare invasive with vaginal-colonising isolates, the results obtained support this conclusion. It has been shown previously that the virulent ST17 clone corresponds to serosubtype III-2 [12], and that it is associated particularly with LO neonatal sepsis, but rarely causes invasive disease in adults [8]. The present, much larger, study confirms a significant association between serosubtype III-2 and LO, but not EO, disease in infants, suggesting significant differences in pathogenesis between these two types of neonatal GBS disease.

Serosubtype III-2 is distinguished easily from the more common serosubtype III-1, which contains IS*1548* and IS*1381*, but not GBSi1, usually belongs to ST19, is a common vaginal coloniser, and is equally common in cases of EO and LO infant and adult GBS disease [8,12]. Although these two major MS III subtypes have antigenically similar polysaccharide capsules, there are consistent *cps* sequence differences that allow them to be distinguished [19].

Meningitis is more common among infants with LO disease than in other groups, but this does not wholly explain the strong association between serosubtype III-2 and LO disease. However, the possibility cannot be excluded that some cases of meningitis were not identified because lumbar punctures were not performed, or because blood, but not CSF, isolates were referred for serotyping. The present study demonstrates that serosubtype III-2 is significantly less likely than other molecular serotypes and serosubtype III-1 to carry AR-associated genes, especially macrolide or aminoglycoside resistance genes. Macrolide resistance is uncommon in Australasia, and further investigation is needed to determine

whether this is also true in regions in which the overall incidence of AR is higher.

The relatively low overall incidence (8%) of AR in the present study was similar to that reported in some previous studies (8–11%) [7,20–23], but lower than that reported in others (16–30%) [7,24–32]. A recent increase in antibiotic resistance among GBS in the USA was associated with the emergence and spread of a single serotype V clone [25], in which macrolide resistance was significantly more common than in other serotypes. Although the prevalence of macrolide and aminoglycoside resistance genes in MS V was moderately high (21%) in the present study, it was not significantly higher than in serosubtype III-1 (18%) or MS II (11%). Neither macrolide nor aminoglycoside resistance markers were identified among New Zealand isolates collected before 1998, but *ermB* was found with increasing frequency between 2001 and 2004, and was associated with increases (not individually statistically significant) in MS V and serosubtype III-1. In Australia, there was no significant difference between the two periods.

On the basis of the present data, a tetravalent Ia, Ib, III, V vaccine could potentially provide protection against 90% of invasive infections in infants, and 87% of those in WCBA in Australia and New Zealand [33]. Alternatively, the use of common protein antigens with a limited number of group-specific polysaccharides would increase the protective range [34].

The mPCR/RLB method used in this study is simple, inexpensive, reproducible, objective and specific. Virtually all isolates are typeable with mPCR/RLB, whereas a significant proportion (11%) of isolates in this study, as in previous studies, were non-typeable or difficult to type by conventional serotyping [35,36]. The present study provided data concerning serotype and AR distribution among invasive GBS isolates in Australasia, and demonstrates the utility of this simple typing method, which would be suitable for routine surveillance in other countries.

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